U.S. EPA
NCEA
M.D. B243-01
(109 T.W. Alexander Dr.)
Research Triangle Park, NC 27711

Phone # (919) 541-4130
Fax # (919) 685-3330

MEMO DATE: September 5, 2018

To: Matthew Himmelstein

DuPont Haskell Global Centers
Newark, DE 19714

Subject: In Vitro Studies of Chloroprene Metabolism

From: Paul M. Schlosser, U.S. EPA

This memo is to request additional information and clarification regarding experimental studies conducted at DuPont Haskell Global Centers (previously Haskell Laboratory for Health and Environmental Sciences) to measure the oxidative metabolism of chloroprene (CP) using microsomes derived from liver and lungs of mice, rats, and humans, as described in Himmelstein et al. (2004) and Yang et al. (2012). The information (data) will assist in evaluation of the resulting mathematical models of the in vitro system, the parameters from which are being considered for use in a chloroprene PBPK model.

There are three data elements that I am seeking, described from highest to least priority.

1) Data regarding the mass-transfer resistance for movement of chloroprene between the headspace and the incubation medium

   One possibility are data demonstrating that under conditions of highest CP metabolic activity (concentrations below metabolic saturation) the rate of metabolism is linear with microsomal content; i.e., data showing the initial rate of clearance from the headspace doubles when the microsomal content is doubled or reduced by 50% when microsomal content is likewise reduced.

   Alternately, data where the rate of partitioning of CP between the headspace and incubation medium has been measured, starting immediately after the CP is introduced to the vial, until air-

medium equilibrium is reached. Such data are distinct from those used to determine the rate of system loss in the absence of metabolic activity after equilibrium is reached.

2) Clarification on headspace sample volumes used in the Himmelstein et al. (2004) study

In Himmelstein et al. (2004), p. 19, right column, last few lines, the methods description indicates that the head-space sample size for the CP oxidation experiments was 400 μL. In the computational scripts received the sample volumes for the corresponding experiments were either set to 200 μL for the (male) mouse and rat liver and lung experiments or to 385.8 μL for the human liver lung. However, in the code provided as part of the 2010 report (IISRP-17520-1388) it appears that the later volume was used for all experiments with male tissues; i.e., from the 2004 paper

a) Please provide clarification on the sample volume(s) used for these various experiments. It makes sense that a single injection volume (~ 400 μL) was used for all experiments described in the 2004 paper, but that this was reduced to 200 μL in the subsequent analysis (female mouse and rat plus all kidney data).

b) Please provide information indicating the precision of the measurement sample sizes if other than one significant figure; i.e., if it was determined to be exactly 200.0 μL for data reported in Yang et al. (2012) but exactly 385.8 μL for data reported in Himmelstein et al. (2004).

3) Incubation vial volumes used in the Himmelstein et al. (2004) study

From the report for studies performed in 2010, IISRP-17520-1388, the vial volumes (weight of water required to fill the vials was ~ 11.65 or 11.63 g. However, in the model scripts provided the vial volume for the human tissue experiments was set to 0.0119573 L (11.9573 g).

In the code provided as part of the 2010 report (IISRP-17520-1388) it appears that the later volume was used for all experiments with male tissues; i.e., from the 2004 paper. This makes sense and lacking other information we will simply use 0.0120 L for all such samples.

If there are data to indicate the male mouse and rat studies conducted for the 2004 paper used vials that were 0.0116 L instead of 0.0120, please provide that information.